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NMR investigation of the complexation and chiral discrimination of pyrazole sulfonamide derivatives with cyclodextrins

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ABSTRACT

The complexes formed between six original chiral diaryl-pyrazole sulfonamide derivatives, displaying poor solubility, and various CDs (native α-, β- and γ-CDs, hydroxypropylated HP-β-CD, methylated Me-β-CD or amino NH_2-β-CD) were studied by 1D and 2D ^{1}H NMR at physiological pH in order to determine their apparent binding constant, stoichiometry and structure of the supramolecular assembly. For some complexes, the spectra obtained for free racemic compound and for racemic compound in presence of CD indicate a splitting of signal(s). Additional experiments with pure enantiomer and enriched enantiomer allow us to attribute this behavior to chiral discrimination. The complexing ability of the native β-CD towards our compounds appears the most promising since binding values around 7×10^2 M^{-1} are obtained. The two-dimensional ROESY (^{1}H–^{1}H) experiments prove the inclusion of the aliphatic part of the compound in the CD cavity. It is noteworthy that this inclusion occurs via the smaller opening of the cavity.

1. Introduction

Cyclodextrins (CDs) are macrolytic compounds with several D-glucopyranose units linked by α-1,4-glycosidic bonds. The common α-CD, β-CD and γ-CD are composed of 6, 7 and 8 glucose units, respectively. The shape of CDs is a truncated cone with a central cavity due to the chair conformation of the glucopyranose units. Their exterior surface is hydrophilic due to the presence of hydroxyl groups whereas the central cavity is lined by skeletal carbons and ethereal oxygens of the glucose residues which gives it a relatively lipophilic character. These properties make them complexing agents of first interest since they are able to form inclusion complexes with a great variety of molecules of appropriate polarity and size (Duchêne, 1987). Their pharmaceutical applications rely mainly on their abilities to enhance the solubility, the stability and the bioavailability of drug molecules (Loftsson & Duchêne, 2007).

CDs are optically active and offer the potential discrimination of enantiomeric substances. They are widely used for the enantioseparation of drugs using high-performance liquid chromatography (Subramanian, 1994) and capillary electrophoresis (Chankvetadze, 1997). The formation of the diastereoisomeric species (between the CD and the enantiomers) is generally described as a result of the inclusion of an apolar part of the guest in the hydrophobic cavity and polar interactions with the outer hydrophilic rim of the CD. To permit enantiodiscrimination, the substituents of the stereogenic center must interact with the hydroxyl groups at the mouth of the CD or/and with the functional group of a derivatized CD (Xiao & Armstrong, 2004). Numerous functionalized CD derivatives are available to modify their complex forming ability and enantioselectivity as methylated-, hydroxypropylated-, sulfated- or amino-CDs for example.

Previously, a new class of human carbonic anhydrase (hCA) inhibitors, diaryl-pyrazole sulfonamide derivatives, has been synthesized and pharmacologically evaluated (Rogez-Florent et al., 2013). These compounds have a very limited water solubility which can limit their pharmaceutical development. Various formulation techniques can be applied to overcome the low aqueous solubility of drugs. Among them, the complexation with CDs offers the possibility to improve their solubility without affecting their original structure and have proved to be one of the most effective (Loftsson & Duchêne, 2007). Moreover, these compounds having a chiral center, it was essential to separate their enantiomers and verify their

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optical purities before envisaging the study of their pharmacological activity. In a previous work, a capillary electrophoretic method was developed in a dual CD mode using the native β-CD and the cationic NH_2-β-CD (Rogez-Florent et al., 2014). Thus, two distinct objectives in the development of our original compounds requires the use of CDs: the enhancement of their aqueous solubility and the separation of their enantiomers. For these objectives, the study of the complexes formed between our compounds and various CDs is essential, either to choose the most appropriate CD or to improve the knowledge on enantiodiscrimination mechanism of our compounds.

The NMR spectroscopy is one of the most useful techniques to study interactions of cyclodextrins with guest compounds (Bakkour et al., 2006; Chankvetadze, Endresz, Schulte, Bergenthal, & Blaschke, 1996; Chankvetadze et al., 1999, 2000a,b; Hellriegel et al., 2001; Zhou et al., 2003a,b; Danel et al., 2006, 2008, 2011; Brun Malta et al., 2008; Bednarek, Bocian, & Michalska, 2008; Marçon et al., 2009; Negi & Singh, 2013; Srinivasan & Stalin, 2014; Yuan, Jin, & Xu, 2012). This technique permits a better description of the supramolecular assembly, especially the right orientation of the guest molecule inside the cavity, and also provides information on the stoichiometry and binding constant of the host:guest complexes (Fielding, 2000).

In this paper, we investigate by 1D and 2D ^{1}H NMR the complexes formed between six original chiral diaryl-pyrazole sulfonamide derivatives and six CDs (native α-, β- and γ-CDs, hydroxypropylated HP-β-CD, methylated Me-β-CD or amino NH_2-β-CD) at physiological pH. The experiments are performed using the racemic or the pure enantiomers of the compounds in order to obtain information on the chiral discrimination. Whereas the stoichiometry is investigated for one complex (using the continuous variation method), the apparent binding constants are determined for the eleven complexes (using the titration method) in order to select the most promising CD. Last, the structure of the supramolecular assembly is investigated with the selected CD.

2. Experimental

2.1. Chemicals

Deionized water was obtained from Milli-*Q* system (Millipore, Saint-en-Yvelines, France). Sodium dihydrogen phosphate and disodium hydrogen phosphate were purchased from Merck (Nogent-sur-Marne, France). Deuterium oxide (100%) and dimethylsulfoxide-*d6* (99.8%) were purchased from Euriso-top (Gif sur Yvette, France).

2.2. Studied compounds

The diaryl-pyrazole sulfonamide derivatives (**1**–**6**, Fig. 1) were designed and synthesized by some of us (Rogez-Florent et al., 2013). The enantiomers of **1** were resolved and prepared by chiral SFC (supercritical fluid chromatography) coupled to a diode array detector using an AD-H column (Daicel®, 250 mm × 10 mm; 5 μm) (others experimental conditions: 30% methanol, 35 °C, 150 bars). The optical rotation of solutions (10 mg/mL in methanol/dichloromethane—33%/67%) were measured using the Na D line (589 nm): $[\alpha]_D^{20}$ = +18 and −18.

2.3. Cyclodextrins

α-CD and γ-CD were purchased from Wacker Chimie (Lyon, France) and 6-monodeoxy-6-monoamino-β-CD (NH_2-β-CD) from Cyclolab (Budapest, Hungary). β-CD, HP-β-CD and Me-β-CD were kindly supplied by Roquette Laboratories (Lestrem, France). The HP-β-CD and Me-β-CD represent multicomponent mixtures with molar substitution (MS) of 0.61 and 0.57 per glucose unit, respectively. Their molar concentration was calculated taking into account their averaged molecular weight.

2.4. Nuclear magnetic resonance

The NMR experiments were realized on a Bruker AVANCE 500 with a TXI probe operating at 500.13 MHz for proton and 125.8 MHz for carbon.

Attributions of the ^{1}H signals of the compounds **1**–**6** were realized by standard NMR experiment spectra: COSY (homonuclear scalar correlation ^{1}H–^{1}H), HSQC (heteronuclear correlation ^{1}H–^{13}C) and HMBC (long range correlation ^{1}H–^{13}C). ROESY (homonuclear dipolar correlation ^{1}H–^{1}H) experiments spectra were recorded with a mixing time of 500 ms. Five hundred microliter of solutions were introduced into standard 5 mm NMR tubes and the experiments were realized at 298 K. The 67 mM phosphate buffer pD 7.4 was prepared by mixing appropriate amounts of both sodium salts in D_2O. Due to the poor solubility of our compounds, all solutions were prepared adding 30% (v/v) of DMSO-*d6*. The **1**–**6** compounds were racemate, equimolar mixture of both enantiomers (otherwise specified).

2.4.1. Determination of the stoichiometry

The stoichiometry of the **1**-(+)/NH_2-β-CD complex was studied using the continuous variation method (Job, 1928). The total concentration of the interacting species was kept constant at 1 mM. The molar fractions r (r = [**1**-(+)]/([**1**-(+)] + [NH_2-β-CD])) varied in the range 0–1.

2.4.2. Determination of the apparent binding constants

The apparent binding constants were determined for eleven complexes: the complexes formed between **1** and the 6 CDs and the complexes forms between β-CD and the 6 compounds. To be in accordance with the Scott's model (Scott, 1956) and take into account that the equilibrium molar concentration of the CD

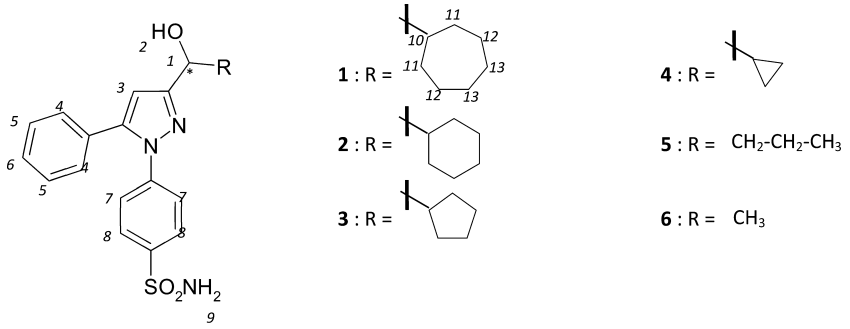

Fig. 1. Structure and assignments of the hydrogen atoms of the studied compounds.